(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
15 September 2005 (15.09.2005)

PCT

(10) International Publication Number
WO 2005/085431 A2

(51) International Patent Classification[7]: C12N 9/10, 15/62, G01N 33/535

(21) International Application Number: PCT/EP2005/050899

(22) International Filing Date: 1 March 2005 (01.03.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
04405123.3 2 March 2004 (02.03.2004) EP
04405465.8 22 July 2004 (22.07.2004) EP

(71) Applicant *(for all designated States except US)*: **EPFL ECOLE POLYTECHNIQUE FEDERALE DE LAUSANNE** [CH/CH]; CM - Ecublens, CH-1015 Lausanne (CH).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BARNIKOV, Jan** [DE/CH]; Delsbergerallee 53, CH-4053 Basel (CH). **CHIDLEY, Christopher** [GB/CH]; Rte. de Marcolet 15A, CH-1023 Crissier (CH). **GRONEMEYER, Thomas** [DE/CH]; Chemin des Perrettes 1, CH-1024 Ecublens (CH). **HEINIS, Christian** [CH/CH]; Kistlerweg 16, CH-3270 Aarberg (CH). **JACCARD, Hughes** [CH/CH]; Cheminet 24, CH-1400 Yverdon-les-Bains (CH). **JOHNSSON, Kai** [DE/CH]; Rue du Midi 20, CH-1003 Lausanne (CH). **JUILLERAT, Alexandre** [CH/CH]; Praz-dom Nicod 10, CH-1000 Lausanne (CH). **KEPPLER, Antje** [DE/CH]; Chemin Vermont 22, CH-1006 Lausanne (CH).

(74) Agent: **BECKER, Konrad**; Aeschenvorstadt 24 P.O. Box 318, CH-4010 Basel (CH).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2005/085431 A2

(54) Title: MUTANTS OF O[6]-ALKYLGUANINE-DNA ALKYLTRANSFERASE

(57) Abstract: The invention relates to AGT mutants showing, when compared to the wild type human AGT, two or more advantageous properties selected from (a) reduced DNA interaction; (b) localisation of the expressed protein in eukaryotic cells that is no longer restricted to the nucleus; (c) improved expression yield as soluble protein and improved stability in various hosts; (d) improved stability under oxidising conditions; (e) improved stability within cells after reaction with a substrate; (f) improved stability outside cells before and after reaction with a substrate; (g) improved *in vitro* solubility; (h) improved reactivity against O^6-alkylguanine substrates; (1) reduced reactivity against DNA-based substrates; and (j) reduced reactivity against N^9-substituted O^6-alkylguanine substrates. Such AGT mutants with the mentioned improved properties are mutants wherein between 1 and 25 amino acids of the wild type human AGT are substituted by other amino acids, and optionally I to 5 amino acids out of the continuous chain at one, two or three positions are deleted or added and/or 1 to 4 amino acids at the N-terminus or 1 to 40 amino acids at the C-terminus are deleted. The invention further relates to a method for detecting and/or manipulating a protein of interest wherein the protein of interest is incorporated into a fusion protein with the AGT mutants of the invention. Another object of the invention are AGT fusion proteins comprising such AGT mutants and the protein of interest.

ATTACHMENT A